Toxicity of an Insecticide to Two Species of Shrimp, *Penaeus aztecus* and *Penaeus setiferus*

by **EDWARD CHIN** *and* **DONALD M. ALLEN**
U. S. Fish and Wildlife Service, Galveston, Texas

In the fall of 1956 mosquito control workers of Galveston County, Texas, received several reports from bait dealers in the Galveston Bay area that several days after aerial spraying of the surrounding marshes with insecticide, considerable mortality of live shrimp occurred in their bait pens. In this area and throughout the Gulf of Mexico live shrimp sold for bait are commonly held in wooden pens suspended in the waters of the bays and bayous. Mortality of shrimp in bait pens attributed by shrimp fishermen to aerial spraying of DDT has been reported in the Ft. Pierce region on the eastern coast of Florida by De Sylva (1954).

Biologists and conservation workers have been concerned with the effects of insecticides on beneficial forms, particularly since the increased use of these chemicals after World War II. Toxicity of various insecticides, especially DDT, to fish and wildlife has been investigated by a large number of workers and excellent references can be found in publications by George, Darsie, and Springer (1957), Cottam (1956), and Doudoroff, Katz, and Tarzwell (1953). With the exception of work by Springer and Webster (1951), Tiller and Cory (1947), and George, Darsie, and Springer (1957), studies have been confined largely to freshwater fishes, birds, and mammals. Little information is available concerning the effect of insecticides on the estuarine flora and fauna. Since considerable aerial spraying is done over salt water marshes, particularly in the South, such studies are especially appropriate.

The insecticide used in the Galveston area is Tri-6 Dust No. 30 (Thompson-Hayward Chemical Company, Kansas City, Missouri). It contains 3.0 per cent gamma isomer of benzene hexachloride, 5.1 per cent other isomers of benzene hexachloride, and 91.9 per cent inert ingredients. Benzene hexachloride and BHC are common names for 1, 2, 3, 4, 5, 6-hexachlorocyclohexane, a chemical frequently used in

commercial insecticides. The material was tested in the experiments described below at the request and with the cooperation of William Cox, Director of the Galveston Mosquito Control District, to determine if it could be considered dangerous to populations of commercially important shrimp.

DETERMINATION OF LETHAL LEVELS

General Methods. Lethal levels were determined for two size groups of shrimp. The larger shrimp ranged from 29 to 50 millimeters in length with a mean of 41.6 millimeters and standard deviation of 5.9. The smaller shrimp ranged from 11 to 13 millimeters in length with a mean of 11.9 millimeters and standard deviation of 0.45. Lengths represent measurements from the tip of the rostrum to the end of the telson. Except where noted, concentrations are in terms of parts per billion (p.p.b.) Tri-6 Dust No. 30. For brevity, this material will be referred to as Tri-6 Dust. Sea water used to make up solutions was filtered through glass wool to remove detritus and macroscopic organisms. Salinities were determined chemically and are in terms of parts per thousand (p.p.t.). All pH readings were obtained at the start of each experiment with a Beckman pH meter. The experiments were conducted in close accordance with the bioassay methods suggested by Doudoroff *et al.* (1951). Due to the nature of the animals, however, certain aspects required modification.

Methods—29 to 50 millimeter size group. Varying quantities of sea water were placed in seven 2-gallon, covered, glass jars, 265 millimeters in height and 230 millimeters in diameter. All solutions were aerated until the experiment was terminated. Air stones used in preliminary experiments showed some evidence of prolonged or permanent contamination with Tri-6 Dust and were replaced with disposable glass pipettes. Six shrimp were placed in each of the seven jars. With the exception of two specimens of *Penaeus aztecus*, all shrimp of this size group were *Penaeus setiferus*. After approximately two hours, additional sea water containing 1000 p.p.b. Tri-6 Dust was added to six of the seven jars to make up seven liters of sea water with resultant concentrations ranging from 2.5 p.p.b. to 100 p.p.b. The seventh jar containing seven liters of sea water was used as a control.

The temperature of the water in the jars remained constant at 26.0° C. throughout the course of the experiment. The salinity of the sea water used to make up the solutions was 31.2 p.p.t. and pH values of the seven solutions ranged from 8.15 to 8.25. Due to the cannibalistic nature of the animals, all were fed with small amounts of fish.

Methods—11 to 13 millimeter size group. The smaller shrimp were exposed to varying concentrations of Tri-6 Dust in a similar manner. The total volume in each two-gallon jar was reduced to four liters and concentrations ranged from 0 to 1500 p.p.b. Previous experience has shown that there is considerable tendency for shrimp of this size to jump out of the water and become fixed to the sides or covers of the jars. To eliminate accidental mortality of this type, the shrimp were placed in 500-milliliter covered, plastic dishes perforated with small holes. Ten small shrimp were placed in each dish and the entire dish was immersed in the four liters of solution. The large number of perforations in the plastic dishes and the agitation of the water due to aeration and activity of the animals permitted a free exchange of fluid between the dish and the surrounding medium.

Temperature of the water in the jars rose gradually from 17.4° C. to 22.3° C. during the experiment. Salinity was 31.4 p.p.t. and pH values of the solutions ranged from 8.15 to 8.2. All shrimp were fed with small quantities of ground veal sold for use as baby food.

Penaeid shrimp less than 15 millimeters in length are difficult to identify and a large sample of the stock from which the experimental animals were drawn was reared to a more advanced stage in the laboratory. This group was comprised entirely of *P. aztecus*.

Results. The results shown in figures 1 and 2 indicate clearly that shrimp are highly sensitive to Tri-6 Dust. In the 29 to 50 millimeter size group mortality was absent in the control jar and at concentrations of 2.5 p.p.b. and 5 p.p.b. At 10 p.p.b. and 25 p.p.b. mortality was negligible. However, at 50 p.p.b. and 100 p.p.b. mortalities of 83 per cent and 100 per cent, respectively, were noted.

In the 11 to 13 millimeter size group mortality was absent in the control jar. At 75 p.p.b. and 125 p.p.b. mortalities of 50 per cent and 80 per cent, respectively, were noted. At all other concentrations tested, 100 per cent mortality occurred.

From the results in figures 1 and 2 differences in sensitivity may exist between the two groups of shrimp. Of the larger shrimp, 83 per cent died at 50 p.p.b. compared to 50 per cent of the smaller shrimp at 75 p.p.b. Differences in sensitivity to the insecticide were demonstrated, also, by a comparison of the estimated 24-hour median tolerance limits. The median tolerance limit (TL_m) or the concentration at which 50 per cent of the test animals are able to survive for a specified period of exposure as suggested by Doudoroff *et al.* (1951) was adopted to derive an index of relative toxicity. Figure 3 shows that the estimated 24-hour TL_m for the smaller shrimp was approximately 400 p.p.b. compared to 35 p.p.b. for the larger shrimp.

Since the larger shrimp were composed almost entirely of *P. setiferus* while the smaller shrimp were composed of *P. aztecus*, differences in sensitivity to Tri-6 Dust could be related either to differences between species or between size groups. Other experiments not reported here indicate that there is no appreciable difference between comparable sizes of the two species in susceptibility to the insecticide. The observed differences, therefore, are attributed to size rather than species differences.

STABILITY OF TOXIC FACTOR

Methods. Since all insecticides have a limited effective life we were interested in the stability of the toxic factor or factors in solution when exposed to sunlight. Four 5-gallon pyrex carboys, containing 14 milligrams of Tri-6 Dust in 14 liters of filtered sea water, were placed outdoors, one every four days for 12 days. The insecticide was added just prior to setting each solution outdoors. Thus, 18 days after the initial carboy was established, we had four solutions, each containing 1000 p.p.b. Tri-6 Dust, that had been exposed to sunlight for 6, 10, 12, and 18 days, respectively. The solutions were tested on the eighteenth day and compared with a fresh solution of equal concentration.

Seven liters of each solution were placed in 2-gallon, covered, glass

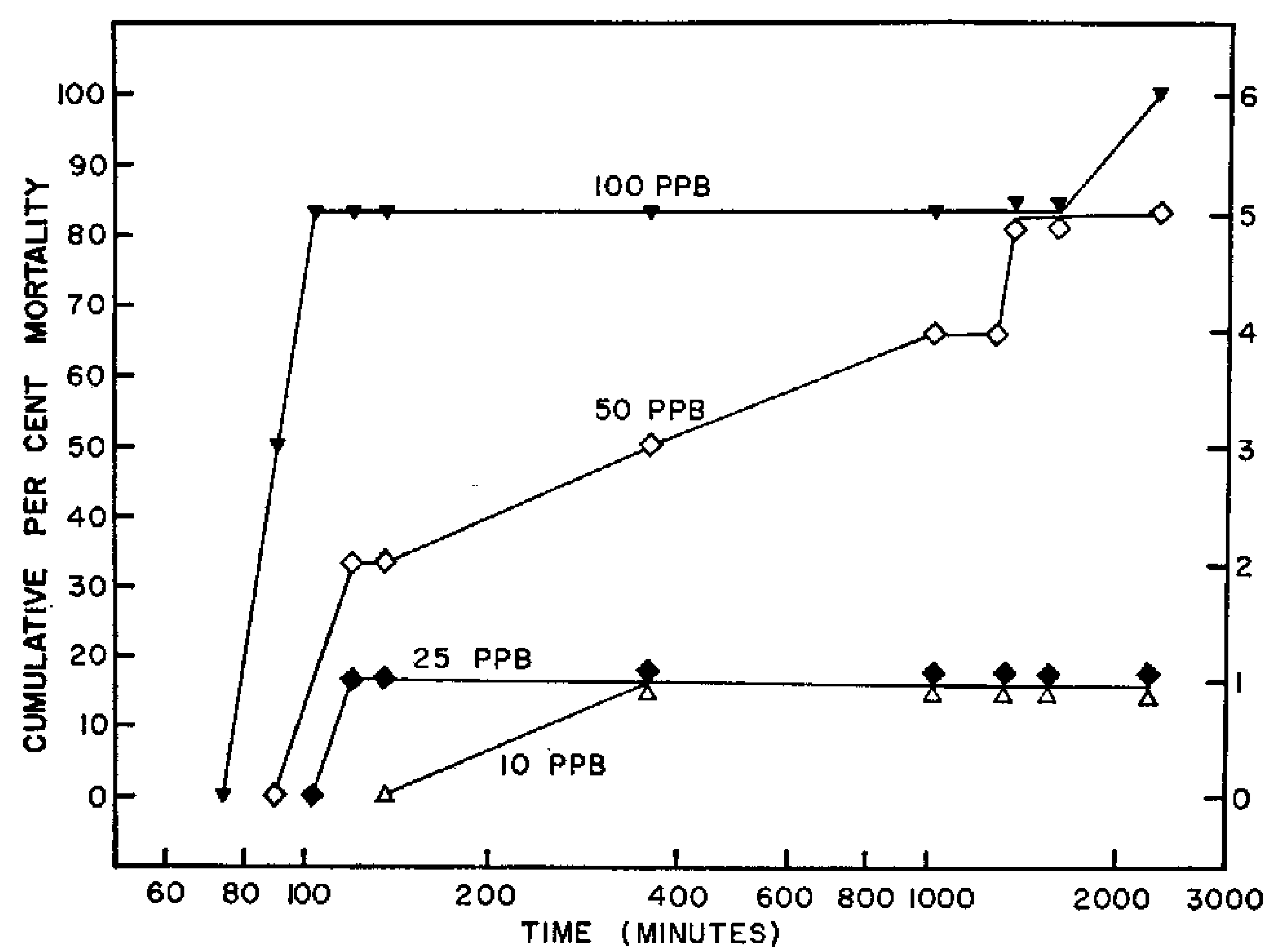

FIG. 1 MORTALITY OF SHRIMP (29-50 MM. SIZE GROUP) AT FOUR CONCENTRATIONS OF TRI-6 DUST.

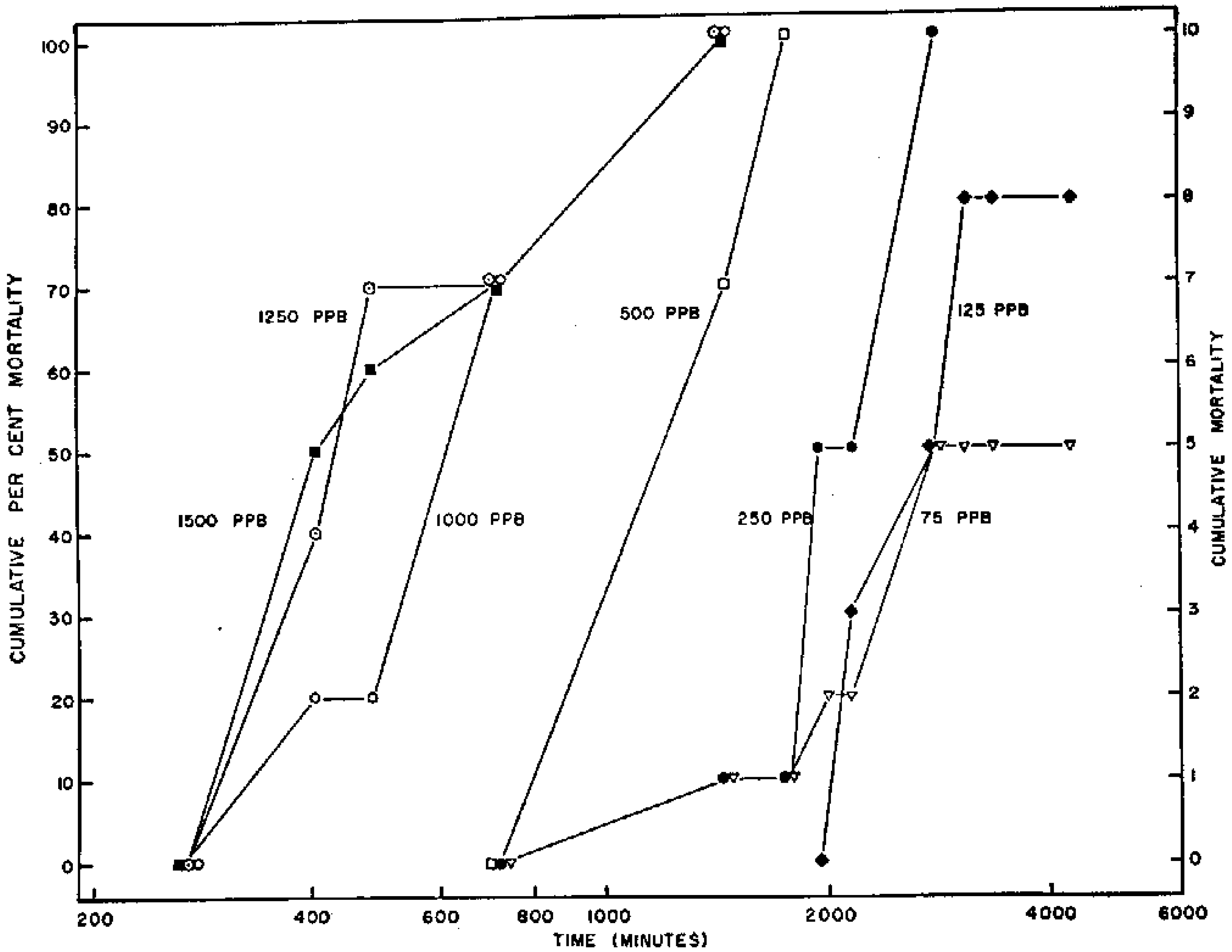

FIG. 2 MORTALITY OF SHRIMP (11-13 MM. SIZE GROUP) AT SEVEN CONCENTRATIONS OF TRI-6 DUST

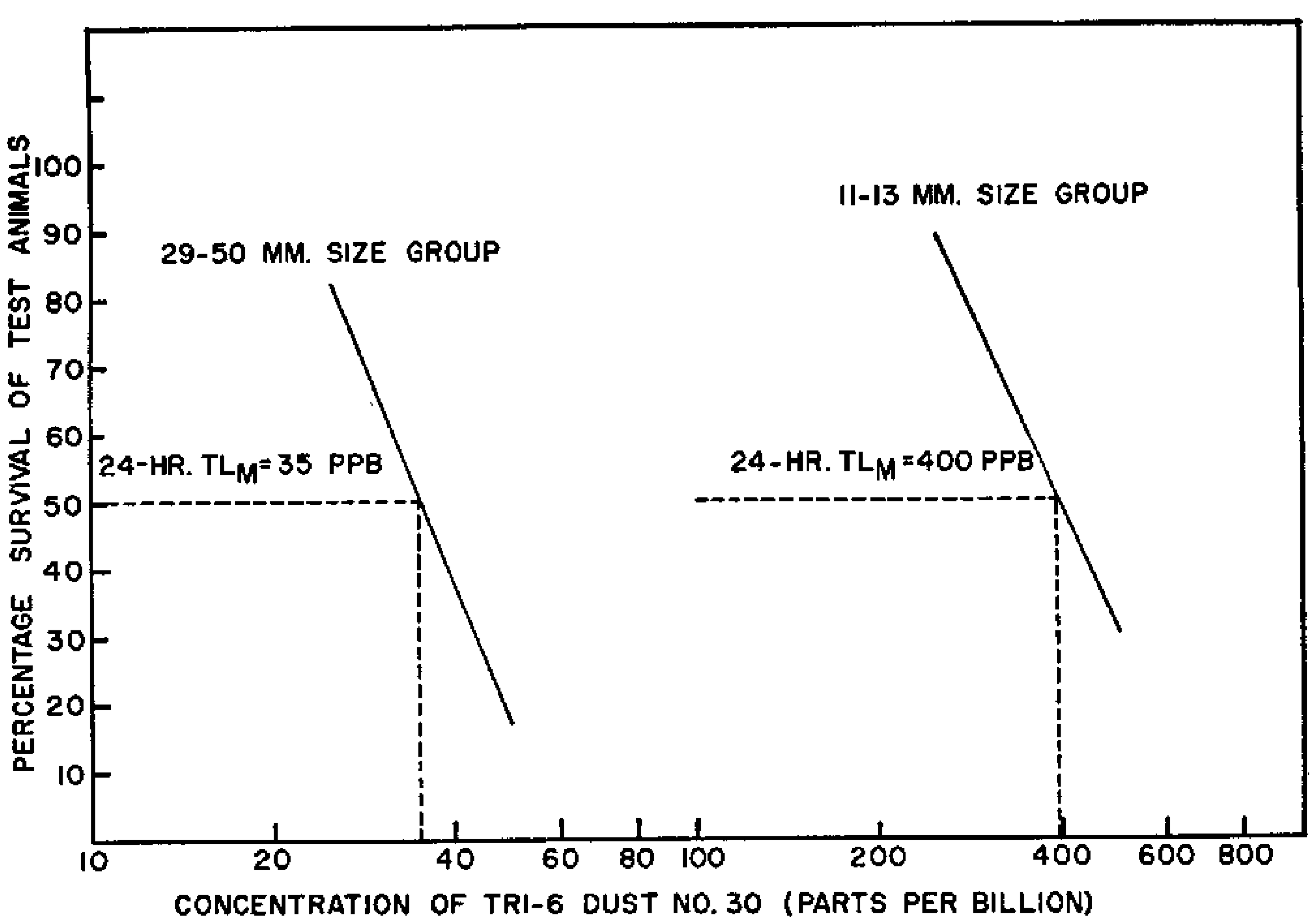

FIG. 3 ESTIMATION OF 24-HR. MEDIAN TOLERANCE LIMITS BY STRAIGHT-LINE GRAPHICAL INTERPOLATION FOR TWO SIZE GROUPS OF SHRIMP.

jars. A control jar was established and six shrimp were immersed in each solution. The shrimp included two species, *P. setiferus* and *P. aztecus*, and ranged in length from 39 to 65 millimeters with a mean of 56 millimeters and standard deviation of 6.4. Water temperatures ranged from 21.5 to 22.5° C. during the experiment. Salinity of the water used to make up the solutions was 31.3 p.p.t. and pH values of the six solutions ranged from 8.0 to 8.2. As in the other experiments, all solutions were aerated.

Results. With the exception of the control group in which no mortality occurred, 100 per cent mortality occurred in all jars within 24 hours. Exposure to sunlight up to 18 days had no apparent effect on the toxicity of the insecticide. Fifty per cent of the test animals at each concentration became moribund within one hour and mortality proceeded at approximately the same rate.

FIELD TEST

A field test was conducted with the cooperation of the Galveston Mosquito Control District. Minnow traps containing shrimp were placed in a bayou near Texas City. The test area received a direct application of Tri-6 Dust from a low flying airplane. Mortality was negligible in all traps. Results were considered inconclusive, however, as the insecticide remained at the surface, and drifted away from the test area through the combined action of the wind and tide. When insecticides are applied over water, it is difficult to evaluate their effects on aquatic organisms without considering tides, currents, wind, depth of water, and other factors.

DISCUSSION AND CONCLUSIONS

Although the field test was inconclusive, results of the laboratory experiments show clearly that Tri-6 Dust can be considered extremely toxic to shrimp. Since 91.9 per cent of the compound consists of inert ingredients, mortalities of 83 per cent of the larger shrimp at 50 p.p.b. and 50 per cent of the smaller shrimp at 75 p.p.b. were caused by actual concentrations of 4 and 6 p.p.b. benzene hexachloride, respectively.

The inverse relationship between size of shrimp and resistance to Tri-6 Dust can be explained, possibly, on the basis of differences in total volume of solution. The smaller shrimp were exposed to a total volume of four liters compared to seven liters for the larger shrimp. Prévost, Lanouette, and Grenier (1948) showed that per cent mortality of fish at a given concentration of DDT increased with an increase in volume of solution. Saila (1953) reported a similar increase in mortality using rotenone as a toxicant. A factor that deserves consideration, however, is the size of the experimental animals. The average weight of the smaller shrimp was .06 gram compared to approximately one gram for the larger shrimp. Thus, on the basis of unit weight the smaller shrimp were exposed to a considerably larger volume of solution.

The stability of the toxic factor or factors requires further investigation. In our experiments, exposure of several 1000 p.p.b. solutions of Tri-6 Dust to sunlight for periods up to 18 days had no apparent effect on the toxicity of the insecticide to shrimp. Since the observed tolerance levels are extremely low, the stability of weaker solutions should be studied.

Evaluation of the effects of insecticides to estuarine organisms under actual conditions is difficult. The Galveston unit has constantly made efforts to avoid dispersing insecticide over water. Nevertheless, fish kills in 15 streams in the Tennessee Valley of Alabama have been attributed by Young and Nicholson (1951) to organic insecticides that were washed into the streams from the soil and vegetation by heavy rains. In seaside areas heavy rainfall and/or a series of high tides could wash deposits of insecticide from the marshes and rice fields into the bayous and estuaries with possible disastrous effects for certain organisms.

Insect control in estuaries and surrounding marshlands presents a problem often faced by entomologists, fishery biologists, and mosquito control workers. These areas are well known breeding grounds for several species of mosquitoes and at the same time are used as nursery grounds by many fish and aquatic invertebrates. Among these are the shrimp tested in the above experiments. Shrimp, presently the country's most valuable fishery product in terms of dollar volume sales, spend several vital months in the bayous and estuaries of the Gulf shortly after hatching. The importance of suggestions and precautions on the application of insecticides by Springer and Haugen (1953), Linduska and Surber (1948) and other workers in the field is obvious.

SUMMARY

Tri-6 Dust No. 30 (Thompson-Hayward Company, Kansas City, Missouri), an insecticide used extensively by mosquito control workers in Galveston County, Texas, was tested in the laboratory to determine if it could be considered toxic to commercially important shrimp. The material contains 3.0 per cent gamma isomer of benzene hexachloride, 5.1 per cent other isomers of benzene hexachloride, and

91.9 per cent inert ingredients. Lethal levels were determined for two size groups of shrimp. The larger shrimp ranged from 29 to 50 millimeters in length and consisted almost entirely of *Penaeus setiferus*. The smaller shrimp ranged from 11 to 13 millimeters in length and consisted entirely of *Penaeus aztecus*. A mortality of 83 per cent at 50 parts per billion was noted for the larger shrimp compared to 50 per cent at 75 parts per billion for the smaller shrimp. The apparent greater sensitivity of the larger shrimp to the insecticide was demonstrated, also, by estimated 24-hour median tolerance limits of 35 parts per billion compared to 400 parts per billion for the smaller shrimp. Differences in sensitivity were attributed to size, rather than species differences. Up to 18 days of exposure to sunlight had no effect on the stability of the toxic factor or factors. A field test was conducted, but results were inconclusive. Nevertheless, laboratory experiments show clearly that Tri-6 Dust No. 30 should be considered extremely toxic to shrimp.

LITERATURE CITED

COTTAM, CLARENCE, 1956—Chemical controls in relation to wildlife. *Virginia Wildlife* 17(10): 12–13, 22–23.

DE SYLVA, DONALD P., 1954—The live bait shrimp fishery of the Northeast Coast of Florida. *Florida Board of Conservation, Technical Series* No. 11: 1–35.

DOUDOROFF, P., B. G. ANDERSON, G. E. BURDICK, P. S. GALTSOFF, W. B. HART, R. PATRICK, E. R. STRONG, E. W. SURBER, AND W. M. VAN HORN, 1951—Bio-assay methods for the evaluation of acute toxicity of industrial wastes to fish. *Sewage and Industrial Wastes* 23(11): 1380–1397.

DOUDOROFF, PETER, MAX KATZ, AND CLARENCE M. TARZWELL, 1953—Toxicity of some organic insecticides to fish. *Sewage and Industrial Wastes*, 25(7): 840–844.

GEORGE, JOHN L., RICHARD F. DARSIE, JR., AND PAUL F. SPRINGER, 1957—Effects on wildlife of aerial applications of Strobane, DDT, and BHC to tidal marshes in Delaware. *Wildlife Man.*, 21(1): 42–53.

LINDUSKA, JOSEPH P., AND EUGENE W. SURBER, 1948—Effects of DDT and other insecticides on fish and wildlife, summary of investigations during 1947. *U. S. Department of Interior, Fish and Wildlife Service, Circular* 15: 1–19.

PREVOST, G., C. LANOUETTE, AND F. GRENIER, 1948—Effect of volume on the determination of DDT or rotenone toxicity of fish. *J. Wildlife Man.* 12(3): 241–250.

SAILA, SAUL B., 1953—Bio-assay procedures for the evaluation of fish toxicants with particular reference to rotenone. *Trans. Am. Fish. Soc.*, 83: 104–114.

SPRINGER, PAUL F., AND ARNOLD O. HAUGEN, 1953—Effect of insecticides on wildlife. *Paper presented at Third Army Annual Insect and Rodent Control Training Conference, Memphis, Tennessee, May* 5–8, 1953. Mimeographed: 1–8.

SPRINGER, PAUL F., AND JOHN R. WEBSTER, 1951—Biological effects of DDT applications on tidal salt marshes. *Mosquito News*, 11(2): 67–74.

TILLER, R. E., AND ERNEST N. CORY, 1947—Effects of DDT on some tidewater aquatic animals. *J. Economic Ent.*, 40(3): 431–433.

YOUNG, L. A., AND H. P. NICHOLSON, 1951—Stream pollution resulting from the use of organic insecticides. *Progressive Fish-Culturist* 13(4): 193–198.